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Genetic characterization of Shiga toxin producing *Escherichia coli* belonging to the emerging hybrid pathotype O80:H2 isolated from humans 2010-2017 in Switzerland

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Abstract

Shiga toxin-producing *E. coli* (STEC) O80:H2 is an uncommon hybrid pathotype that has recently emerged in France. We analysed 18 STEC O80:H2 isolated from humans in Switzerland during 2010-2017. All isolates carried *stx2a* or *stx2d*, the rare *eae* variant *eae*-ξ and at least seven virulence genes associated with pS88, a plasmid that is found in extraintestinal pathogenic *E. coli* (ExPEC). Whole genome sequencing (WGS) identified additional chromosomal extraintestinal virulence genes encoding for type 1 fimbria (*fimA, fimC and fimH*), aerobactin (*iuc*/*iutA*) and afimbrial adhesins (*afaA*/*C*/*D*/*E*-VIII). Core genome multi-locus sequence typing (cgMLST) detected two closely related but distinct subclusters with different *stx2* and *iuc*/*iutA* genotypes. All isolates were multidrug resistant (MDR), but susceptible to third generation cephalosporins and azithromycin. STEC/ExPEC hybrid pathotypes such as STEC O80:H2 represent a therapeutical challenge in the event of extraintestinal infection.

Keywords

STEC O80:H2, extraintestinal, virulence, hybrid, core genome

1. Introduction

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are important foodborne pathogens and responsible for gastrointestinal illnesses which may involve non-bloody or bloody diarrhea, haemorrhagic colitis (HC), and the haemolytic uremic syndrome (HUS) (Karch et al., 2005). The primary virulence trait of STEC is Stx, which includes two major groups, Stx1 and Stx2, whereby Stx2a, Stx2c and Stx2d are mainly associated with severe disease (Fuller et al., 2011). An additional virulence trait that may be present in STEC includes intimin, an outer membrane protein which is responsible for the ability to form attaching and effacing lesions in the human intestinal mucosa (Jerse et al. 1990). Intimin is encoded by the chromosomal gene *eae*, which is part of a pathogenicity island termed the locus for enterocyte effacement, LEE (Kaper et al. 2004). Differentiation of *eae* subtypes represents a valuable tool for typing STEC in the clinical setting as well as for epidemiological studies. At present, 30 distinct *eae* subtypes have been identified and appended by lower case Greek letters and Roman numbers α1, α2, α8, β1, β2, β3, γ1, γ2, ε1, ε2, ε3, ε4, ζ, ζ3, η, η2, θ, ι1, ι2, κ, λ, μ, ν, ξ, ο, π, ρ, σ, τ, and υ, respectively (Ooka et al. 2012). *E. coli* O157:H7 is reportedly the most common STEC serotype in the European Union and in Switzerland, nonetheless, non-O157 STEC serogroups, in particular O26, O91, O103, O111, O121 and O145, are also frequently detected (EFSA, 2017; Fierz et al. 2007). By contrast, reports of STEC O80:H2 strains are rare. However, this pathotype has recently emerged in France and is associated with severe cases of HUS, as well as HUS associated with bacteremia (Mariani-Kurkdjian et al., 2014; Soysal et al., 2016). A further case of STEC O80:H2 induced lethal complication of HUS was very recently reported in the Netherlands (Wijnsma et al., 2017). This unusual STEC serotype features the rare eae-ξ (xi), and genetic determinants encoded by the pS88 plasmid which is associated with extraintestinal-virulence pathogenic *E. coli* (ExPEC) (Peigne et al., 2009).

This study aimed to examine the molecular characteristics of 18 human STEC O80:H2 isolates collected during 2010-2017 at the National Centre for Enteropathogenic Bacteria and *Listeria* (NENT) in Zürich, Switzerland, using conventional PCR methods and whole genome sequencing. Moreover, the genetic relatedness of the strains was determined using core genome multilocus sequence typing.

2. Materials and Methods

2.1. Bacterial strains

For this study, we analysed 18 STEC O80:H2 human isolates received between 2010 and 2017 at the NENT in Zürich, Switzerland. Ten strains (55.6%) were from female, and eight (44.4%) from male patients. The median age was 28 years (range <1 – 81 years). Six (33.3%) strains were isolated from patients ≤5 years of age. Twelve (66.6%) of the infections occurred during the summer–early autumn season. The majority (n=13, 72.2%) of the cases were registered in the western parts of Switzerland that share borders with the high-incidence regions of France (Soysal et al., 2016). Aggregate clinical data was attainable for 10 patients. Thereof, one (10%) developed HUS, and four (40%) were hospitalised.

2.2. Ethics statement

All the clinical isolates were collected from stool samples in the course of diagnostic procedures and were processed at the NENT. This study was approved by the local ethics committee of Zürich (BASEC-Nr.Req-2016-00374).

90 **2.3. Serotyping**

91 The O80 serogroup was determined by O80-specific PCR using primers and conditions
92 described previously (Soysal et al., 2016). The H2 type was identified by PCR targeting the
93 *flic*$_{H2}$ gene with primers described elsewhere (Alonso et al., 2017).

94

95 **2.4. Detection of virulence genes**

96 The presence of *stx* genes was initially determined by real-time PCR (LightCycler
97 R 2.0 Instrument, Roche Diagnostics Corporation, Indianapolis, IN, USA) (EURL, 2013a).
98 PCR-based identification of *stx1* and *stx2* subtypes was carried out as described in a previous
99 study (Scheutz et al., 2012). The presence of *eae* and the identification of the *eae*-ξ variant
100 was verified using methods described previously (Blanco et al., 2005; EURL, 2013a). The
101 strains were further screened by PCR for the presence of *hlyA* encoding enterohemolysin
102 (Schmidt et al., 1995), *iha*, encoding an iron acquisition protein (Schmidt et al., 2001), the
103 subtilase cytotoxin gene, *subAB* (Funk et al., 2013), *ipaH*, characteristic for enteroinvasive *E.*
104 *coli* (EIEC) (Persson et al., 2007), *aggR* coding for a transcriptional regulator in
105 enteroaggregative *E. coli* (EAEC) (EURL, 2013b), and the pS88 related genes *sitA*, *eitB, cia,*
106 *iss, iucC*, *iroN*, *hlyF*, *etsC*, *cvaA*, and *ompT*$_p$ (Peigne et al., 2009).

107

108 **2.5. Multi locus sequence typing (MLST)**

109 MLST was performed by PCR amplification of internal fragments of seven housekeeping
110 genes (*adk*, *fumC*, *gyrB*, *icdF*, *mdh*, *purA*, and *recA*) (Wirth et al., 2006). Custom sequencing
111 of the alleles was performed by Microsynth (Balgach, Switzerland). Sequence types (STs)
112 were assigned in accordance with the *E. coli* MLST database website
113 (https://pubmlst.org/databases.shtml).

114

115 **2.6. Whole genome sequencing (WGS) and *in silico* analysis**

116 Whole genome sequencing was performed using a MiSeq Illumina platform with 2x 300nt
117 pair-end sequencing as previously described (Meinel et al., 2014). Reads were *de novo*
118 assembled using SPAdes (version 3.11.1) (Bankevich et al., 2012) and the resulting assembly
119 was polished using Pilon (version 1.22) (Walker et al., 2014). Mean coverage of the
120 sequenced genomes was more than 50-fold.
121 We carried out *in silico* genome analysis using the virulence factor database (VFDB) (Chen
122 et al., 2005), to determine the presence of virulence genes. Furthermore, we performed a core
123 genome MLST to assess the genetic relatedness among the isolates. The core genome MLST
124 is based on ATCC 25922 and was generated using Ridom SeqSphere Software (version 4.1.9,
125 available at http://www.ridom.de/seqsphere/cgmlst/).
126 Antimicrobial resistance genes were searched for using the RGI tool (version 3.2.1) that is
127 based on the CARD database (Jia et al., 2017).
128

129 **2.7. Antimicrobial susceptibility testing**

130 Antimicrobial susceptibility testing was performed using the disk-diffusion method and the
131 antibiotics ampicillin (AM), amoxicillin-clavulanic acid (AMC), cefazolin (CZ), cefotaxime
132 (CTX), cefepime (FEP), nalidixic acid (NA), ciprofloxacin (CIP), gentamicin (GM),
133 kanamycin (K), streptomycin (S), sulfamethoxazole/trimethoprim (SXT), fosfomycin (FOS),
134 azithromycin (AZM), nitrofurantoin (F/M), chloramphenicol (C) and tetracycline (T) (Becton
135 Dickinson, Heidelberg, Germany). Results were interpreted according to Clinical and
136 Laboratory Standards Institute (CLSI) performance standards (CLSI, 2016). For
137 azithromycin, an inhibition zone diameter of ≤ 12 mm was considered resistant. Multidrug
138 resistance (MDR) was defined as resistance to three or more classes of antimicrobials,
139 counting ß-lactams as one class.

3. Results

3.1 Detection of virulence genes

Of the 18 STEC O80 strains, nine (50%) harboured *stx2a*, and 9 further (50%) *stx2d* (Table). All isolates harboured the rare variant of the intimin gene, *eae*-ξ. Fourteen isolates encoded *hlyA*, and 13 *iha*, respectively (Table). All 18 isolates contained at least seven pS88-related virulence genes (Table).

In silico genome analysis revealed that all 18 isolates carried fimbria associated genes *fimA, fimC* and *fimH* (Table). Further, 9 isolates contained the aerobactin encoding genes *iucA*, *iucB*, *iucC*, and *iutA*. Finally, *afa*-VIII genes encoding for afimbrial adhesins were detected in three isolates (Table).

3.2 Clonal relationship among the STEC O80:H2 isolates

MLST by PCR assigned all 18 isolates to ST301. Using core genome data, we identified two distinct but highly related clusters of the STEC O80:H2-ST301 strains (Table and Figure). Cluster 1 consisted of nine isolates that harboured *stx2a* (Figure). Cluster 2 contained nine isolates that contained *stx2d* (Figure). Furthermore, cluster 2 consisted of the isolates containing the *iucA*, *iucB*, *iucC*, and *iutA* genes, and contained the three isolates carrying *afa*-VIII genes (Table and Figure). Finally, in contrast to isolates from cluster 1, all isolates belonging to cluster 2 harboured pS88 associated *etsC* (Table).

3.3. Antimicrobial susceptibility

Antimicrobial drug susceptibility testing revealed that all strains were MDR, i.e., resistant to three or more classes of antimicrobials, counting ß-lactams as one class (Supplementary Material Table 1). Rates of resistance were 100% for ampicillin, streptomycin, and

sulfamethoxazole/trimethoprim. Fourteen (77.8%) of the isolates were resistant to nalidixic acid, 13 (72.2%) to tetracycline, and nine (50%, all belonging to cgMLST cluster 2) to chloramphenicol. None of the isolates were resistant to third-generation cephalosporins, ciprofloxacin, fosfomycin, azithromycin or nitrofurantoin (Supplementary Material Table 1). In correlation to the phenotypic profiles, the genotypical presence of bla_{TEM-1} and *aph(6)-Id* was confirmed *in silico* for all isolates, whereas *sul-1*, *sul-2*, genes were detected in 16 isolates (Supplementary Material Table 2). The RGI resistance gene tool did not detect any genes that predict resistance to tetracycline or chloramphenicol.

3.4. GenBank accession numbers

DNA sequences are available under the accession numbers PYSA00000000 to PYSF00000000, and PYRO00000000 to PYRZ00000000, respectively.

4. Discussion

In this study, we characterised 18 STEC O80:H2 isolates that were collected from humans during 2010-2017 in Switzerland. This rare serotype has been described as an emerging STEC in eastern regions of France, and the demographic characteristics of the patients in this study are suggestive of a possible common source or route of infection. However, the source of this serotype has so far not been identified (Soysal et al., 2016).

STEC O80:H2 is associated with high extraintestinal virulence potential, due to the presence of virulence genes encoded on plasmid pS88. This plasmid was first detected in neonatal meningitis *E. coli (*NMEC) O45:H7 ST95, a major etiological agent of meningitis and urosepsis in infants in France (Bonacorsi et al., 2003). The pS88 sequence comprises several virulence regions homologous to plasmids pAPEC-O2-ColV and pAPEC-O1-ColBM from avian pathogenic *E. coli* (APEC) O2:K1 and O1:K1, strains that cause colibacillosis in

chicken (Johnson et al., 2006a; Johnson et al., 2006b). Peigne et al. (2009) have demonstrated that this plasmid sustains high level bacteremia in the neonatal rat model and that pS88-like plasmids are widely distributed among MNEC clones, uropathogenic *E. coli* strains (UPEC), and avian pathogenic E. coli strains (APEC), including *E. coli* O18, O1, O2 and O83 and *E. coli* belonging mostly to ST95. By contrast, STEC O80:H2 appears so far to be the only instance of pS88 found among *E. coli* belonging to ST 301. Nevertheless, within ST301 a number of further *E. coli* serogroups type have been registered in the EcMLST databank and described in the literature, including an STEC O4:H– strain from diarrheic calves (Wieler et al., 1996), an *E. coli* O5 strain from a human infection (Gangiredla et al., 2017), NMEC O7 (Peigne et al., 2009), clinical isolates *E. coli* O132:H2 and O55:H9 (Chattaway et al., 2017), STEC O180:H2 (Joensen et al., 2014), and *E. coli* O186 (Weimer, 2017). Whether any of these strains harbour pS88, and whether ST301 serogroups represent a particular genetic background for the acquisition of pS88-like plasmids remains to be elucidated.

Moreover, it remains unclear to what extent pS88 may be involved in the pathogenicity of other STEC/ExPEC strains described previously. Such hybrids predominantly include UPEC/STEC hybrid strains such as the urovirulent O2:H6 ST141 (Bielaszewska et al., 2014), strains involved in HUS associated with urinary tract infections (UTI), including O157:H7, O17:H18, O103:H2, O174:H2, O145:H28, and O5:H–, some of which lacked identifiable uropathogenic virulence factors such as *papA* (Starr et al., 1998). STEC/ExPEC strains involved in bacteremia have been described for STEC O128ab:H2 (Buvens et al., 2013), O157:H2 (Chiurchiu et al., 2003), and O138:H– (Nguyen et al., 2007), however, characterisations of the genetic factors involved in extraintestinal pathogenicity are lacking.

In addition to pS88, the STEC O80:H2 isolates in this study harboured further genes implicated in extraintestinal virulence. All isolates carried *fimA, fimC* and *fimH*, genes involved in biosynthesis of type 1 fimbria which are crucial for *E. coli* adhesion to epithelial

host cells as well as intracellular survival in phagocytes (Avalos Vizcarra et al., 2016). Furthermore, some of isolates containing the *iucA*, *iucB*, *iucC*, and *iutA*. These genes are involved in the biosynthesis of aerobactin, an iron uptake system that is associated with pathogenesis in extraintestinal *E. coli* strains and frequently present in EAEC clinical isolates, including the 2011 hybrid STEC/EAEC O104:H4 outbreak strain in Germany (Garcia-Angulo et al., 2013). A minority of the isolates carried *afa*-VIII genes encoding for afimbrial adhesins, which are present in both diarrheal and uropathogenic *E. coli* strains and also widespread among bovine pathogenic *E. coli* strains associated with diarrhoea and septicaemia (Antão et al., 2009). Notably, EPEC O80:H2 has recently been identified as an emerging pathogen in young calves and could be a precursor of STEC O80:H2 (Thiry et al., 2017). Further characterisation of these isolates would be desirable in order to establish any common virulence traits between human and calf strains and to attempt an identification the source of STEC O80:H2.

Taken together, our findings provide further evidence for the high pathogenicity and the extraordinary hybrid STEC/ExPEC characteristics which distinguishes STEC O80:H2 from other STEC serotypes.

Although the strains in this study are closely related, cgMLST indicated a trend of subclonal divergence into distinct clusters with different virulence genotypes. While both clusters 1 and 2 include strains carrying *stx2* variants associated with severe disease (*stx2a* or *stx2d*), and genes for type 1 fimbria, cluster 2 comprises strains with potentially higher extraintestinal virulence due to the additional virulence gene *etsC* encoded on pS88, the presence of aerobactin genes *iuc*/*iutA*, and in some cluster 2 isolates, the afimbrial adhesion genes *afaA*/*C*/*D*/*E*-VIII. These data suggest a genetic plasticity of STEC O80 regarding the acquisition of extraintestinal virulence factors. Notably, as opposed to the STEC O80:H2 strains isolated in France (Soysal et al., 2016), none of the strains from this study harboured

stx2a/2d or *stx2c/2d* combinations. Moreover, in France, 74% of the STEC O80:H2 harboured *stx2c/2d* and these accounted for the majority (56.9%) of the HUS cases. Among the 10 patients for whom clinical data was available in this study, one (10%) had HUS. The absence of the *stx2c/2d* genotype among the strains isolated in Switzerland may account for the lower prevalence of HUS.

The clinical significance of extraintestinal virulence potential is exemplified by a case report of STEC O80:H2 associated bacteraemia (Mariani-Kurkdjian et al., 2014), raising the controversial question about antibiotic therapy during invasive STEC infection (Freedman et al., 2016). Antimicrobial drug susceptibility testing revealed that all strains were multidrug resistant (MDR), i.e., resistant to three or more classes of antimicrobials (Supplementary Material Table 1). Nevertheless, all isolates remained susceptible to third-generation cephalosporins and azithromycin. Our data therefore lend support to a therapeutical approach suggested by Soysal et al. (2016), which involves the combination of ceftriaxone with azithromycin to treat invasive infections of STECO80:H2. This hypervirulent, MDR hybrid pathotype exemplifies the need to monitor antimicrobial resistance in STEC as well as in other *E. coli* pathotypes. Finally, STEC O80:H2 may represent a threat in terms of public health. Surveillance and characterization of STEC isolates from severe cases of human disease using culture-based methods and WGS to supplement non-culture methods such as PCR based *stx* detection may improve the identification and source tracking of STEC O80:H2 infections.

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Conflicts of interest

None to declare.

References

Alonso, C. A., Mora, A., Díaz, D., Blanco, M., González-Barrio, D., Ruiz-Fons, F., Simón, C., Blanco, J., Torres, C., 2017. Occurrence and characterization of *stx* and/or *eae*-positive *Escherichia coli* isolated from wildlife, including a typical EPEC strain from a wild boar. Vet. Microbiol. 207, 69-73. doi=10.1016/j.vetmic.2017.05.028.

Antão, E. M., Wieler, L. H., Ewers, C., 2009. Adhesive threads of extraintestinal pathogenic *Escherichia coli*. Gut Pathog. 1, 22. DOI=10.1186/1757-4749-1-22.

Avalos Vizcarra, I., Hosseini, V., Kollmannsberger, P., Meier, S., Weber, S. S., Arnoldini, M., Ackermann, M., Vogel, V., 2016. How type 1 fimbriae help *Escherichia coli* to evade extracellular antibiotics. Sci. Rep. 6, 18109. doi=10.1038/srep18109.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., Pevzner, P. A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455-477. doi=10.1089/cmb.2012.0021.

Bielaszewska, M., Schiller, R., Lammers, L., Bauwens, A., Fruth, A., Middendorf, B., Schmidt, M. A., Tarr, P. I., Dobrindt, U., Karch, H., Mellmann, A., 2014. Heteropathogenic virulence and phylogeny reveal phased pathogenic metamorphosis in *Escherichia coli* O2:H6. EMBO Mol. Med. 6, 347-357. DOI=10.1002/emmm.201303133.

Blanco, M., Schumacher, S., Tasara, T., Zweifel, C., Blanco, J. E., Dahbi, G., Blanco, J., Stephan, R., 2005. Serotypes, intimin variants and other virulence factors of *eae* positive *Escherichia coli* strains isolated from healthy cattle in Switzerland. Identification of a new intimin variant gene (*eae*-η2). BMC Microbiol. 5:23. doi: 10.1186/1471-2180-5-23.

298 Bonacorsi, S., Clermont, O., Houdouin, V., Cordevant, C., Brahimi, N., Marecat, A., Tinsley,
299 C., Nassif, X., Lange, M., Bingen, E. 2003. Molecular analysis and experimental
300 virulence of french and north american *Escherichia coli* neonatal meningitis isolates:
301 Identification of a new virulent clone. J. Infect. Dis. 187, 1895–1906.
302 Buvens, G., De Rauw, K., Roisin, S., Vanfraechem, G., Denis, O., Jacobs, F., Scheutz, F.,
303 Piérard, D., 2013. Verocytotoxin-producing *Escherichia coli* O128ab: H2 bacteremia in a
304 27-year-old male with hemolytic-uremic syndrome. J. Clin .Microbiol. 51, 5, 1633-1635.
305 Chattaway, M. A., Schaefer, U., Tewolde, R., Dallman, T. J., Jenkins, C., 2017. Identification
306 of *Escherichia coli* and *Shigella* species from whole-genome sequences. J. Clin.
307 Microbiol. 55, 616-623.
308 Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., Jin, Q., 2005. VFDB: a reference
309 database for bacterial virulence factors. Nucleic Acids Res. 33, Database issue, D325-8.
310 doi=10.1093/nar/gki008.
311 Chiurchiu, C., Firrincieli, A., Santostefano, M., Fusaroli, M., Remuzzi, G., Ruggenenti, P.,
312 2003. Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin
313 *Escherichia coli* O157:H7 bacteremia and urinary tract infection. Am. J. Kidney Dis. 41,
314 1, E4. DOI=10.1053/ajkd.2003.50022.
315 CLSI. 2016. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-sixth
316 supplement, CLSI Document M100S. Clinical and Laboratory Standards Institute,
317 Wayne.
318 EFSA (European Food Safety Authority) and ECDC (European Centre for Disease
319 Prevention and Control), 2017. The European Union summary report on trends and
320 sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J.
321 2017;15(12):5077, 228 pp. https://doi.org/10.2903/j.efsa.2017.5077.

322 EURL (European Union Reference Laboratory). 2013a. Identification and characterization
323 of Verocytotoxin-producing *Escherichia coli* (VTEC) by real time PCR amplification of
324 the main virulence genes and the genes associated with the serogroups mainly associated
325 with severe human infections. EU-RL VTEC_Method_02_Rev 0. available at:
326 http://old.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_02_Rev_0.pdf.
327 EURL (European Union Reference Laboratory). 2013b. Detection of enteroaggregative
328 *Escherichia coli* in food by real time PCR amplification of the *aggR* and *aaiC* genes. EU
329 RL_Method_05_Rev 1. available at:
330 http://old.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_05_Rev_1.pdf.
331 Fierz , L., Cernela, N., Hauser, E., Nüesch-Inderbinen, M., Stephan, R., 2017. Human
332 infections with Shiga toxin-producing *Escherichia coli*, Switzerland, 2010-2014. Front.
333 Microbiol. 8:1471. doi: 10.3389/fmicb.2017.01471.
334 Freedman, S. B., Xie, J., Neufeld, M. S., Hamilton, W. L., Hartling, L., Tarr, P. I., Alberta
335 Provincial Pediatric Enteric Infection Team, A. P. P. E. T. I. T. E., Nettel-Aguirre, A.,
336 Chuck, A., Lee, B., Johnson, D., Currie, G., Talbot, J., Jiang, J., Dickinson, J., Kellner,
337 J., MacDonald, J., Svenson, L., Chui, L., Louie, M., Lavoie, M., Eltorki, M., Vanderkooi,
338 O., Tellier, R., Ali, S., Drews, S., Graham, T., Pang, X. L., 2016. Shiga toxin-producing
339 *Escherichia coli* infection, antibiotics, and risk of developing hemolytic uremic
340 syndrome: A meta-analysis. Clin. Infect. Dis. 62, 10, 1251-1258.
341 doi=10.1093/cid/ciw099.
342 Fuller, C. A., Pellino, C. A., Flagler, M. J., Strasser, J. E., Weiss, A. A., 2011. Shiga toxin
343 subtypes display dramatic differences in potency. Infect. Immun. 79, 3, 1329-1337.
344 doi=10.1128/IAI.01182-10.

345 Funk, J., Stoeber, H., Hauser, E., Schmidt, H., 2013. Molecular analysis of subtilase
346 cytotoxin genes of food-borne Shiga toxin-producing *Escherichia coli* reveals a new
347 allelic *subAB* variant. BMC Microbiol. 13, 230. doi=10.1186/1471-2180-13-230.
348 Gangiredla, J., Mammel, M. K., Barnaba, T. J., Tartera, C., Gebru, S. T., Patel, I. R.,
349 Leonard, S. R., Kotewicz, M. L., Lampel, K. A., Elkins, C. A., 2017. Species-wide
350 collection of *Escherichia coli* isolates for examination of genomic diversity. Genome
351 Announc. 5, 50, e01321-17.
352 Garcia-Angulo, V. A., Farfan, M. J., Torres, A. G., 2013 Hybrid and potentially pathogenic
353 *Escherichia coli* strains, in: Donnenberg, M. (ed.), *Escherichia coli*. 2nd ed. Academic
354 Press, Cambridge MA, pp. 331-359.
355 Jia, B., Raphenya, A. R., Alcock, B., Waglechner, N., Guo, P., Tsang, K. K., Lago, B. A.,
356 Dave, B. M., Pereira, S., Sharma, A. N., Doshi, S., Courtot, M., Lo, R., Williams, L. E.,
357 Frye, J. G., Elsayegh, T., Sardar, D., Westman, E. L., Pawlowski, A. C., Johnson, T. A.,
358 Brinkman, F. S., Wright, G. D., McArthur, A. G., 2017. CARD 2017: expansion and
359 model-centric curation of the comprehensive antibiotic resistance database. Nucleic
360 Acids Res. 45, D1, D566-D573. doi=10.1093/nar/gkw1004.
361 Jerse, A. E, Yu, J., Tall, B. D., Kaper, J. B., 1990. A genetic locus of enteropathogenic
362 *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue
363 culture cells. Proc. Natl. Acad. Sci. 87, 7839–7843.
364 Joensen, K. G., Scheutz, F., Lund, O., Hasman, H., Kaas, R. S., Nielsen, E. M., Aarestrup, F.
365 M., 2014. Real-time whole-genome sequencing for routine typing, surveillance, and
366 outbreak detection of verotoxigenic *Escherichia coli*. J. Clin. Microbiol. 52, 1501-1510.
367 DOI=10.1128/JCM.03617-13.

368 Johnson, T.J., Johnson, S.J., Nolan, L.K. 2006b. Complete DNA sequence of a ColBM
369 plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely
370 related colv virulence plasmids. J. Bacteriol. 188, 5975–5983.
371 Johnson, T.J., Siek, K.E., Johnson, S.J., Nolan, L.K. 2006a. DNA sequence of a ColV
372 plasmid and prevalence of selected plasmid-encoded virulence genes among avian
373 *Escherichia coli* strains. J. Bacteriol. 188, 745–758.
374 Kaper, J.B., Nataro, J., Mobley, L.T., 2004. "Pathogenic *Escherichia coli*." Nat. Rev.
375 Microbiol. 2, 123–140.
376 Karch, H., Tarr, P. I., Bielaszewska, M., 2005. Enterohaemorrhagic *Escherichia coli* in
377 human medicine. Int. J. Med. Microbiol. 295, 405-418. doi=10.1016/j.ijmm.2005.06.009.
378 Mariani-Kurkdjian, P., Lemaître, C., Bidet, P., Perez, D., Boggini, L., Kwon, T., Bonacorsi,
379 S., 2014. Haemolytic-uraemic syndrome with bacteraemia caused by a new hybrid
380 *Escherichia coli* pathotype. New Microbes New Infect. 2, 127-131.
381 doi=10.1002/nmi2.49.
382 Meinel, D. M., Margos, G., Konrad, R., Krebs, S., Blum, H., Sing, A., 2014. Next generation
383 sequencing analysis of nine *Corynebacterium ulcerans* isolates reveals zoonotic
384 transmission and a novel putative diphtheria toxin-encoding pathogenicity island.
385 Genome Med. 6, 113. doi=10.1186/s13073-014-0113-3.
386 Nguyen, Q. V., Hochstrasser, L., Chuard, C., Hächler, H., Regamey, C., Descombes, E.,
387 2007. Adult hemolytic-uremic syndrome associated with urosepsis due to Shigatoxin-
388 producing *Escherichia coli* O138:H-. Ren. Fail. 29, 747-750.
389 DOI=10.1080/08860220701460418.
390 Ooka, T., Seto, K., Kawano, K., Kobayashi, H., Etoh, Y., Ichihara, S., Kaneko, A., Isobe, J.,
391 Yamaguchi, K., Horikawa, K., Gomes T.A.T., Linden, A., Bardiau, M., Mainil, J.G.,

392 Beutin, L., Ogura, Y., Hayashi, T. (2012). Clinical significance of *Escherichia albertii*.
393 Emerg. Infect. Dis. 18, 488-492.
394 Peigne, C., Bidet, P., Mahjoub-Messai, F., Plainvert, C., Barbe, V., Médigue, C., Frapy, E.,
395 Nassif, X., Denamur, E., Bingen, E., 2009. The plasmid of *Escherichia coli* strain S88
396 (O45: K1: H7) that causes neonatal meningitis is closely related to avian pathogenic *E.*
397 *coli* plasmids and is associated with high-level bacteremia in a neonatal rat meningitis
398 model. Infect. Immun. 77, 2272-2284.
399 Persson, S., Olsen, K. E., Scheutz, F., Krogfelt, K. A., Gerner-Smidt, P., 2007. A method for
400 fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine
401 diagnostic laboratory. Clin. Microbiol. Infect. 13, 516-524. doi=10.1111/j.1469-
402 0691.2007.01692.x.
403 Scheutz, F., Teel, L. D., Beutin, L., Piérard, D., Buvens, G., Karch, H., Mellmann, A.,
404 Caprioli, A., Tozzoli, R., Morabito, S., Strockbine, N. A., Melton-Celsa, A. R., Sanchez,
405 M., Persson, S., O'Brien, A. D., 2012. Multicenter evaluation of a sequence-based
406 protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J. Clin.
407 Microbiol. 50, 2951-2963. doi=10.1128/JCM.00860-12.
408 Schmidt, H., Beutin, L., Karch, H., 1995. Molecular analysis of the plasmid-encoded
409 hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. 63, 1055-1061.
410 Schmidt, H., Zhang, W.-L., Hemmrich, U., Jelacic, S., Brunder, W., Tarr, P. I., Dobrindt, U.,
411 Hacker, J., Karch, H., 2001. Identification and characterization of a novel genomic island
412 integrated at *selC* in locus of enterocyte effacement-negative, Shiga toxin-producing
413 *Escherichia coli*. Infect. Immun. 69, 6863-6873.
414 Soysal, N., Mariani-Kurkdjian, P., Smail, Y., Liguori, S., Gouali, M., Loukiadis, E., Fach, P.,
415 Bruyand, M., Blanco, J., Bidet, P., Bonacorsi, S., 2016. Enterohemorrhagic *Escherichia*

coli hybrid pathotype O80:H2 as a new therapeutic challenge. Emerg. Infect. Dis. 22, 1604-1612. doi=10.3201/eid2209.160304.

Starr, M., Bennett-Wood, V., Bigham, A. K., de Koning-Ward, T. F., Bordun, A. M., Lightfoot, D., Bettelheim, K. A., Jones, C. L., Robins-Browne, R. M., 1998. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli*: case report and review. Clin. Infect. Dis. 27, 310-315.

Thiry, D., Saulmont, M., Takaki, S., De Rauw, K., Duprez, J. N., Iguchi, A., Piérard, D., Mainil, J. G., 2017. Enteropathogenic *Escherichia coli* O80:H2 in young calves with diarrhea, Belgium. Emerg. Infect. Dis. 23, 2093-2095. DOI=10.3201/eid2312.170450.

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., Earl, A. M., 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One. 9, e112963. doi=10.1371/journal.pone.0112963.

Weimer, B. C., 2017. 100K Pathogen Genome Project. Genome Announc. 5, 28, 10.1128/genomeA.00594-17.

Wieler, L. H., Vieler, E., Erpenstein, C., Schlapp, T., Steinrück, H., Bauerfeind, R., Byomi, A., Baljer, G., 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of *eae* and other genes. J. Clin. Microbiol. 34, 2980-2984.

Wijnsma, K. L., Schijvens, A. M., Rossen, J. W. A., Kooistra-Smid, A. M. D. M., Schreuder, M. F., van de Kar, N. C. A. J., 2017. Unusual severe case of hemolytic uremic syndrome due to Shiga toxin 2d-producing *E. coli* O80:H2. Pediatr. Nephrol. 32, 1263-1268. doi=10.1007/s00467-017-3642-3.

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., Karch, H., Reeves, P. R., Maiden, M. C. J., Ochman, H., Achtman, M., 2006. Sex and virulence in *Escherichia*

441 *coli*: an evolutionary perspective. Mol. Microbiol. 60, 1136-1151. doi=10.1111/j.1365-
442 2958.2006.05172.x.
443
444
445

Table: Genetic backgrounds of 18 Shiga toxin producing *Escherichia coli* serotype O80:H2-ST301, Switzerland, 2010-2017.

Gene*	Description of gene product	No.(%) isolates	cgMLST[1] cluster
EHEC associated markers			
stx2a	Shiga toxin variant	9 (50)	1
stx2d	Shiga toxin variant	9 (50)	2
*eae-*ξ	Intimin variant (attaching and effacing protein)	18 (100)	1, 2
hlyA	Enterohemolysin	14 (77.8)	1, 2
iha	Iron acquisition protein	13 (72.2)	1, 2
subAB	Subtilase cytotoxin	0 (0)	–
EIEC associated marker			
ipaH	Invasion plasmid antigen	0 (0)	–
EAEC associated marker			
aggR	Transcriptional activator of aggregative adherence fimbria I	0 (0)	–
ExPEC associated markers			
sitA	Periplasmic iron transport protein	18 (100)	1, 2
eitB	*E. coli* iron transport protein	0 (0)	–
cia	Colicin Ia (bacteriocin)	17 (94.4)	1, 2
iss	Increased serum survival protein	18 (100)	1, 2
iroC	ATP binding cassette	18 (100)	1, 2
iroN	Salmochelin siderophore receptor	18 (100)	1, 2
hlyF	Hemolysin	18 (100)	1, 2
etsC	Putative type I secretion outer membrane protein	9 (50)	2
cvaA	Colicin V secretion protein	18 (100)	1, 2
ompTp	Outer membrane protease (omptin)	18 (100)	1, 2
fimA	Type-1 fimbrial protein	18 (100)	1, 2
fimC	Chaperone protein for the biogenesis of type 1 fimbriae	18 (100)	1, 2
fimH	Type 1 fimbrial adhesion	18 (100)	1, 2
iucA	Aerobactin siderophore biosynthesis enzyme	9 (50)	2
iucB	Aerobactin siderophore biosynthesis enzyme	9 (50)	2
iucC	Aerobactin siderophore biosynthesis enzyme	9 (50)	2
iutA	Outer membrane receptor for the ferric-siderophore complex	9 (50)	2
afaA-VIII	Afimbrial adhesion	3 (16.7)	2
afaC-VIII	Afimbrial adhesin usher protein	3 (16.7)	2
afaD-VIII	Afimbrial invasion	3 (16.7)	2
afaE-VIII	Afimbrial adhesion	3 (16.7)	2

*EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EAEC, enteroaggregative *E. coli*; ExPEC, extraintestinal pathogenic *E. coli*.

[1]core genome multilocus sequence type.

Figure legend

Figure: Core genome multilocus sequence type (cgMLST) based minimum spanning tree of 18 human Shiga toxin producing *Escherichia coli* (STEC) O80:H2-ST301 isolates. Each circle contains the strain ID(s). Year of isolation is indicated in square brackets. Blue circles represent the *stx-2a* genotype, red circles indicate the *stx2d* genotype. Cluster 1 is shaded in light green. Cluster 2 is shaded in light yellow and contains strains with the *iuc*/*iutA* genotype. Strains within cluster 2 with the *afa*-VIII genotype are shaded in lime. The numbers on connecting lines represent the number of allelic differences between two strains.